IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:		§	
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	TONY PELED	§	
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Serial No.:	10/774,843	§	
		§	
Filed:	February 09, 2004	§	Group Art Unit: 1633
		§	
For:	EXPANSION OF RENEWABLE STEM CELL POPULATIONS	§	
		§	
		§	Attorney Docket: **26691**
Examiner: LEAVITT, MARIA GOMEZ, PhD		§	

Commissioner for Patents
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DECLARATION OF DR. TONY PELED UNDER 37 CFR §1.132

I, Tony Peled, am the Chief scientist and vice president of Gamida-Cell Ltd. at Jerusalem, Israel. My professional specialization is in the field of cell biology, particularly with regard to stem cell culture and therapeutic applications. A copy of my curriculum vitae with some of the most recent publications has been made of record.

I am the Inventor of the present invention. I have read the present application and the new and amended claims, as well as the Office Action from the Examiner and the accompanying references. In support of the accompanying Response to this Office Action, I set forth below some important experimental data which demonstrates some of the unique characteristics of the claimed invention.

I have read and understood the relevant portions of the Office Action dated September 25, 2008 ("Office Action"), and I understand that the Examiner rejected claim 401, 411, 412, 414, 416-419, 422-424, 437, 438 and 466 of the present invention directed to: *a method of ex-vivo expanding a population of CD34+ hematopoietic stem cells by (a) culturing said CD34+ stem cells ex-vivo under conditions allowing for cell proliferation, and (b) at the same time, culturing said cells in the presence of exogenously added nicotinamide, nicotinamide analog or nicotinamide* as being anticipated by, and rendered obvious in view of Brown et al (US Patent No: 5,945,337). I have also read the Brown et al. publication. I have read the Examiner's comment that "*Brown R teaches the requirements for the basal medium composition for expansion of CD34+/CD38- cells ex-vivo <u>including nicotinamide</u> at concentration of 4 mg/L. Though Brown et al., does not specifically disclose that expansion of CD34+ substantially*

inhibits differentiation, substantial inhibition of differentiation is implicitly necessary, absent evidence to the contrary, as the culturing conditions in Brown are the same."

I would like to indicate that at the time of the present application (2004) the use of nicotinamide for *ex-vivo* expansion of hematopoietic stem cells was not known in the art. Indeed, nowhere in Brown is there any mention, suggestion or implication that the presence or concentration of nicotinamide is in any way associated with, critical to or important to stem cell expansion. Thus, not only does Brown not consider nicotinamide concentration essential to the claimed culture medium and conditions, or worthy of "optimization", but Brown is clearly oblivious to any importance of nicotinamide for *ex-vivo* expansion and inhibition of differentiation of hematopoietic stem cells.

In stark contrast to the Examiner's allegations, I maintain that *ex-vivo* culture of hematopoietic stem cells in the presence of cytokines and nicotinamide at the range of concentrations allegedly disclosed in Brown et al. (4 mg/L, equivalent to 0.033 mM nicotinamide), or theoretically up to 10 times as much (0.33 mM nicotinamide) would not be capable of supporting expansion while substantially inhibiting differentiation of the hematopoietic stem cells.

The results of the hematopoietic stem cell culture described hereinbelow provide conclusive evidence that addition of nicotinamide at the concentrations allegedly taught by Brown et al. is insufficient to support significant expansion and substantial inhibition of differentiation of undifferentiated populations of *ex-vivo* cultured hematopoietic stem cells for at least three weeks duration of culture.

As an inventor of the present application, and director of the laboratory at Gamida-Cell, Ltd., I either performed myself or caused to be performed the experiment described in greater detail below, along with graphical representation of the results.

Expansion of hematopoietic stem and progenitor cells fraction by culture of hematopoietic cells with 0.0 mM to 5.0 mM nicotinamide.

Hematopoietic cells maintained in the presence of cytokines and nicotinamide showed continued increased expansion of the CD34+/CD38- subpopulation at 3 weeks duration of culture. The experiments themselves are described in greater detail below.

Mononuclear cell fraction collection and purification:

Human blood cells were obtained from umbilical cord blood from female patients following full-term, normal delivery (informed consent was obtained). Samples were collected and processed within 12 hours postpartum. Blood was mixed with 3 % Gelatin (Sigma, St. Louis, MO), sedimented for 30 minutes to remove most red blood cells. The leukocyte-rich fraction was harvested and layered on a Ficoll-Hypaque gradient (1.077 gram/ml; Sigma), and centrifuged at 400 *g* for 30 minutes. The mononuclear cell fraction in the interface layer was collected, washed three times and resuspended in phosphate-buffered saline (PBS) solution (Biological Industries) containing 0.5 % bovine serum albumin (BSA, Fraction V; Sigma).

Purification of CD133$^+$ cells from mononuclear cell fractions:

To purify CD133$^+$ mononuclear cells, the fraction was subjected to two cycles of

immuno-magnetic separation using the MiniMACS® or Clinimax® CD34 Progenitor Cell Isolation Kit (Miltenyi Biotec, Auburn, CA) as per manufacturer's recommendations. The purity of the $CD133^+$ population obtained ranged from 95 % to 98 % as was determined by flow cytometry.

Ex-vivo expansion of $CD133^+$ cell populations:

$CD133^+$ expressing purified cells above were cultured in 24-well Costar Cell Culture Clusters (Corning Inc., Corning, NY) or culture bags (American Fluoroseal Corp), at a concentration of 10^4 cells/ml in alpha medium (Biological Industries, Beit Haemek, Israel) supplemented with 10 % fetal bovine serum (FBS, Biological Industries). The following human recombinant cytokines were added: Thrombopoietin (TPO), interleukin-6 (IL-6), FLT-3 ligand and stem cell factor (SCF), all at final concentrations of 50 ng/ml each. All cytokines used were purchased from Perpo Tech, Inc. (Rocky Hill, NJ). Nicotinamide was added to cell cultures at concentrations of 0.0, 0.05, 0.1, 0.33, 2.5 or 5 mM for three weeks culture period.

The cultures were incubated at 37 °C, 5 % CO_2 in a humidified atmosphere.

At weekly intervals, cell cultures were topped up with fresh medium or semi-depopulated and were supplemented with fresh medium, serum and cytokines. At predetermined time points, cells were harvested, stained with trypan blue, counted, and cell morphology was determined via the use of cytospin (Shandon, UK)-prepared smears stained with May-Grunwald/Giemsa solutions.

Surface antigen analysis:

Cells were harvested, washed with a PBS solution containing 1 % bovine sera albumin (BSA) and 0.1 % sodium azide (Sigma), and stained at 4 °C for 60 minutes with fluorescein isothiocyanate or phycoerythrin-conjugated antibodies (all from Immunoquality Products, the Netherlands). The cells were then washed with the same buffer and analyzed by FACS caliber or Facstarplus flow cytometers. Cells were passed at a rate of 1000 cells/second, using saline as the sheath fluid. A 488 nm argon laser beam served as the light source for excitation. Emission of ten thousand cells was measured using logarithmic amplification, and analyzed using CellQuest software. Negative control staining of cells was accomplished with mouse IgG-PE (Dako A/S Glostrup, Denmark) and mouse IgG-FITC (BD Biosciences, Erembodegem, Belgium).

Determination of the fraction of early hematopoietic progenitor cells, $CD34^+/CD38^-$ cells following ex vivo expansion:

The cultured cells were washed with PBS/1%BSA, and stained (at 4°C for 30 min) with FITC anti-CD38 and PE anti-CD34 for determination of CD34+CD38- cell percentages (Becton Dickinson) as described above. The fraction positive for CD34 and negative for CD38 was defined as $CD34^+CD38^-$

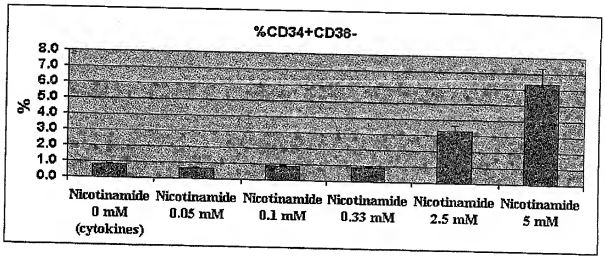
RESULTS

Table 1 illustrates the expansion of the $CD34^+/CD38^-$ hematopoietic cells in three week cultures treated without (0.0) or with increasing concentrations of nicotinamide, as indicated. Figure 1 is a histogram representing the average values from three independent experiments.

Table 1-Expansion of $CD34^+/CD38^-$ subsets from $CD133^+$ Cells

Group	%CD34+CD38-			Average	S.E
	1	2	3		
Nicotinamide 0.0 mM (cytokines only)	0.76	0.81	0.88	0.82	0.060
Nicotinamide 0.05 mM	0.74	0.73	0.53	0.67	0.068
Nicotinamide 0.1 mM	0.95	0.94	0.83	0.91	0.038
Nicotinamide 0.33 mM	0.91	0.96	0.8	0.89	0.047
Nicotinamide 2.5 mM	3.09	3.93	2.9	3.31	0.316
Nicotinamide 5 mM	4.95	8.32	5.84	6.37	1.008

FIGURE 1



Note the clear absence of any discernible effect of nicotinamide at concentrations from 0.05 to 0.33 mM on expansion of the hematopoietic stem and progenitor population ($CD34^+/CD38^-$ cells), over 3 weeks, and the significant expansion of the $CD34^+/CD38^-$ subpopulation in the presence of 2.5 mM and 5 mM nicotinamide, as compared with cytokines alone (nicotinamide 0.0 mM).

As a person signing below, I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may

jeopardize the validity of the Application or any patent issued thereon.

December 24, 2008

Dr. Tony Peled